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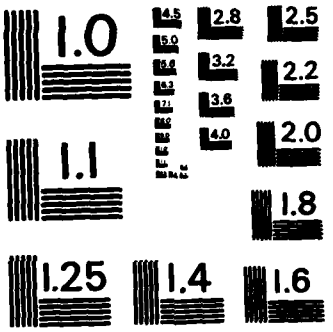

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

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18. SUPPLEMENTARY NOTES

Enclosed, in addition to final report, four manuscripts (in press):
1.) "Clonal Analysis of HLA-DR and -DQ associated determinants - their contribution to Dw specificities"; Reinsmoen, N.L., Bach, F.H. Hum. Immunol., 1985, in press. (cont.)

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Class II molecules
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DNA sequence

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The sequence polymorphism underlying T lymphocyte recognition as related to the DR alpha beta dimer has been studied using cDNA libraries. DR beta genes from homozygous typing cells expressing the same serologically-defined DR antigen but differing in their Dw subtype, have allowed the pinpointing of single amino acid differences underlying this allotypic variation in some combinations, and two to three amino acid differences in others.

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Block 18 (cont.)

2.) "DNA and protein studies of HLA class II molecules: their relationship to T cell recognition"; Segall,M., Cairns,J.S., Dahl,C.A., Curtsinger,J.M., Freeman,S., Nelson,P.J., Cohen,O., Wu,S., Nicklas,J.N., Noreen,H.J., Linner,K.M., Saunders,T.L., Choong,S.A., Ohta,N., Reinsmoen,N.L., Alter,B.J., and Bach,F.H.; Immunol. Rev.; In press.

3.) "HLA-D: The T cell perspective"; Bach,F.H., Ohta,N., Anichini,A., and Reinsmoen,N.L.; in: Human Class II Histocompatibility Antigens. (Eds: S.Ferrone, B.Solheim, E.Moller); Springer-Verlag. 1985, In press.

4.) "Sequence polymorphism of HLA-DR β1 alleles relating to T cell-recognized determinants"; Cairns,S., Curtsinger,J.M., Dahl,C.A., Freeman,S., Alter,B.J., and Bach,F.H.; Nature, In press.

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Final Report
N00014-85-0004
"Molecular Approaches to Selective Immunization"

The goals for the contract referenced above were to establish cDNA cloning and sequencing technology that would permit evaluation of sequences from cells differing by certain well-designated Dw specificities on homozygous typing cells (HTCs). The specific scientific question to be answered was to evaluate DNA sequence variation that existed between alleles of a DR β locus (known to be polymorphic) among HTCs that all express the same serologically-defined specificity, but that differed with regard to the specificities recognized by T cells, i.e. defined by HTCs in the primary mixed leukocyte culture (MLC). It was reasoned in the application that differences between such HTCs might be of a lesser extent than those found between DR β sequences from cells expressing different serologically-defined DR antigens, and that this lesser polymorphism might be, thus, more readily related to the sequence variation underlying T cell recognition.

One difficulty that potentially existed in the proposed studies was to differentiate between the two DR β genes that can be expressed in DR4 haplotypes, the particular haplotype that we proposed for study. These are referred to as DR β_1 and DR β_2. It is known that the DR β_2 gene is relatively invariant. Fortunately, based on a number of different lines of investigation, we were able to conclude that alleles we studied were alleles of the DR β_1 locus, the polymorphic locus thought to encode not only the allelism encoding the serologically-defined DR specificities, but also that carrying much of the polymorphism seen by T lymphocytes.

Our results fully answered the first, and fundamental question that we posed for the contract. Namely, we were able to define three alleles of the DR β_1 locus present in HTCs, all of which type as DR4 but express different Dw specificities subtypic to DR4, Dw4, Dw13, and Dw14. Since the differences between alleles were minimal, certain conclusions can be reached for some comparisons.

We used cloning into pUC 9 and sequencing by the dideoxy method. As a modification, we used the sub-cloning technique of Dale and co-workers.

Assignment of our sequences to DR β_1 was based largely on sequences obtained by Spies and co-workers (see reprint attached) at the genomic level, in which they were able to clearly identify DR β_1 and DR β_2 since they were able to compare the DNA sequences with the N-terminal sequences obtained in proteins isolated with antibodies of known β_1 or β_2 specificity. Our sequences were clearly more closely related to the DR β_1 sequence.

We were able to sequence 2 DR β_1 genes, one from an HTC-expressing DR4-Dw14, and one from an HTC-expressing DR4-Dw13. In addition, we were able to identify, using cloned cytotoxic T lymphocytes, the DR components of an HLA haplotype in an HLA heterozygous cell from which Long and co-workers isolated and sequenced a DR β gene. We identified this haplotype as having components of Dw4 and, based on sequence comparisons with the genes that we sequenced, we were able to assign the sequence obtained by Long to the DR β_1 locus as well.

The results, now published in Nature, demonstrate that the three sequences, i.e. from Dw4, Dw13, and Dw14, differ by between 1 and 3 amino acids at amino acid positions 71, 74, and 86. These are, thus, in the N-terminal domain. The sequences for Dw14 and Dw13 are given in the attached reprint. As can be seen, these two sequences differ for only a single base pair substitution that results in a glutamic acid at position 74 in Dw13, and an alanine at the same position in Dw14. The Dw4 sequence of Long and co-workers differs from those obtained by us for Dw13 and Dw14 due to a lysine at amino acid 71 (instead of the arginine present in the Dw14 and Dw13 sequences) and a glycine at position 86 (instead of the valine present in Dw13 and Dw14).

We thus feel we have answered the questions that were posed in the contract.

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